Macromolecules

Article

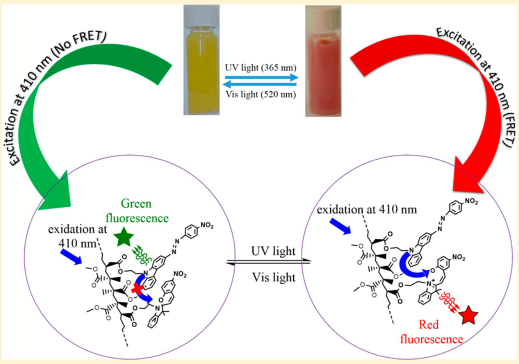
pubs.acs.org/Macromolecules

FRET Phenomenon in Photoreversible Dual-Color Fluorescent Polymeric Nanoparticles Based on Azocarbazole/Spiropyran Derivatives

Jaber Keyvan Rad, Ali Reza Mahdavian,* Hamid Salehi-Mobarakeh, and Amin Abdollahi

Polymer Science Department, Iran Polymer & Petrochemical Institute, P.O. Box 14965/115, 14977-13115 Tehran, Iran

ABSTRACT: Incorporation of chromophores into a polymer chain results in prominent photoreversibility and fatigue resistance, photostability over the long term, and restriction of the internal conversion (IC). Here, we report the copolymerization of two photoactive monomers with methyl methacrylate via emulsion polymerization in order to obtain photoswitchable dual-color fluorescent nanoparticles. For this purpose, azocarbazole ethyl acrylate (AzoCzEA) and spiropyran ethyl acrylate (SPEA) were synthesized and the fluorescence polymeric latex nanoparticles were prepared correspondingly. FT-IR and ^{1}HNMR spectra were used to confirm the structure of novel fluorescent AzoCzEA. UV−vis studies of the obtained nanoparticles displayed the spectral features of both AzoCzEA and SPEA under stimuli-irradiation and inclusion of these chromophores into the polymer particles. DSC analysis revealed an increase in T_g of the prepared copolymer, indicating covalently incorporation of the photoactive monomers into the polymer chains. The optimum ratio of two chromophores to achieve complete quenching and highest energy transfer was determined by UV−vis spectroscopy. DLS and SEM results demonstrated particle size distribution of 40−80 nm with spherical morphology. Fluorescence spectra revealed remarkable fluorescence resonance energy transfer (FRET) from AzoCzEA to SPEA after UV irradiation at 365 nm and dual-color characteristic of the prepared nanoparticles. Besides, an enhancement in the photoreversibility, photostability, prevention of IC, dye leakage, and aggregation were studied elaborately. The obtained results were attributed to the involvement of such chromophores into the polymeric matrix via covalent bonding. Labeling and tracking of living cells and rewriteable patterning are potential applications for such dual-color fluorescent nanoparticles.



1. INTRODUCTION

The fluorescent photoswitchable nanoparticles have received increasing attention because of their exploitations in life science such as selectively highlighting biological substances (e.g., proteins and living cells) and individually light addressable devices.[1,2] Organic photochromic spiropyran derivatives are among the most convenient compounds which have isomeric forms of closed-ring (SP) and the colored conjugated open-ring merocyanine (MC). This isomerization occurs under UV−vis irradiation alternatingly and is widely used due to their fast photochromic response and photoreversible fluorescence modulation through energy transfer.[3−5] Anchoring such photochromic groups covalently into a polymer chain induces their light-sensitive properties. However, light is an especially attractive external stimulus and results in producing light responsive polymers.[6,7] Upon light irradiation, the chemical and physical parameters of the photochromic polymeric nanoparticles would be consequently altered.[8−11]

Polymers containing carbazole moieties are of considerable interest due to their potential applications in highly fluorescent probes and detectors,[12−14] organic light emitting diode materials,[15] hollow particles,[16] photorefractive materials,[17,18] photovoltaic devices,[19] and they are most frequently used as polymeric hosts because of their hole transporting attribute and large band gap.[20] Carbazole derivatives have a long π conjugation that results in high fluorescent emission quantum yields. They can easily be modified by different groups at 3 and/or 6 positions of the carbazole ring to tune the optical and spectroscopic properties. Copolymers containing carbazole derivatives could be used in drug delivery with fluorescence tracing capability and have exhibited negligible cytotoxicity.[12,21,22] Fluorescent commoners can be introduced to polymeric nanoparticles by covalent linkage, entrapment, or self-assembly strategies that make them very promising and versatile for their applications in many systems.[1,23]

The fluorescence resonance energy transfer (FRET) systems consist of a donor and an acceptor dye in which the fluorescence emission of a component can be switched on and off through a nonradiative energy transfer between nearest chromophores (light-sensitive molecules) by UV−vis irradiation with the same excitation wavelengths.[1,24−29] FRET is a dynamic quenching mechanism, because energy transfer occurs while the donor is in the excited state.[30,31] Energy transfer occurs on nanometeric distance and depends on the precise spatial arrangement and

Received: November 4, 2015
Revised: December 7, 2015

DOI: 10.1021/acs.macromol.5b02401
Macromolecules XXXX, XXX, XXX−XXX

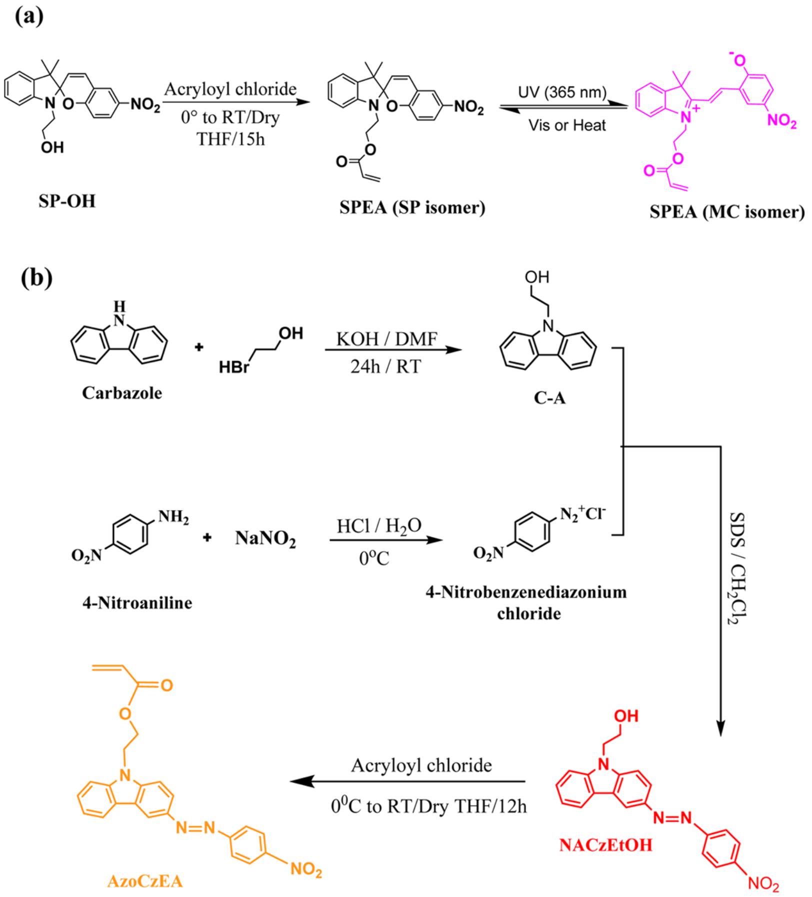


Figure 1. Modification and isomerization of SPEA (a); and synthetic method for preparation of AzoCzEA (b).

approximately parallel transition dipoles in donor and acceptor moieties.[25,26,32] In recent years, materials with optically modulated properties, like fluorescence resonance energy transfer, have attracted much attention as a result of some advantages over conventional fluorescence dyes in broad range of scientific fields including biological diagnosis, cell labeling, imaging, and sensing tools.[23,33]

Photoswitchable dual-color fluorescents are appropriate candidates especially due to their ability to distinguish fake signals made by adventitious fluorescent biomolecules from original ones.[34−36] Zhu and co-workers[37] integrated photoswitchable spyropiran and fluorescent perylene diimide dyes via covalently linkage into a polymeric nanoparticle to generate an optically addressable dual-color fluorescent system by means of emulsion polymerization. The prepared photoluminescent nanoparticles exhibit superior resistance to photobleaching, high contrast, and high intense fluorescence with potential application in biological tracking and labeling. Incorporation of noncovalently bonded chromophores into the nanoparticles may have some disadvantages in long-term stability because of leakage and aggregation of the dyes and decrease in photoresponsivity. Chen et al.[24] reported the reversible modulation of fluorescence by spyropiran and a nitrobenzoxadizole (NBD) derivative dye via miniemulsion polymerization. The spiropyran-based monomer was covalently incorporated into the polymer nanoparticles and doped (noncovalently) with NBD. The efficiency of FRET was primarily good but might have some disadvantages in biological system due to the unbounded NBD dye, leading to FRET weakening and cells cytotoxicity. This group has already synthesized amphiphilic photoswitchable fluorescent polymeric nanoparticles by a facile one-pot RAFT-mediated miniemulsion method.[5] In their research, boron-dipyrromethene phenol methacrylate and spiropyran-linked methacrylate dyes have been covalently incorporated into the polymer backbone. The results exhibited excellent structural stability, prominent photostability, favorable photoreversibility and also lack of potential dye leakage, and aggregation that restrict their biological applications. The as-prepared photoswitchable fluorescent polymeric nanoparticles demonstrated high-contrast fluorescence cell imaging and rewriteable fluorescence pattering successfully. Wang et al.[38] prepared carbazole-spironaphtoxazine copolymers through radical polymerization. The reversibly changes in fluorescence intensity by irradiation of UV−vis light were attributed to the photoinduced electron transfer between carbazole and open-ring form of spirooxazine moiety. The major problem in this work was the absence of adequate overlap between emission spectrum of the donor (carbazole derivative) and absorption spectrum of the acceptor (spironaphtoxazine) dyes for total quenching of the fluorescence intensity under UV irradiation. On the other hand, photostability and fatigue resistance fell due to the accessibility of the donor and acceptor dyes to the surrounding environment. A donor−acceptor of another couple based on spiropyran as a dopant in polyvinyl carbazole has been reported in 2011 for development in erasable holography.[39]

To the best of our knowledge, there is no report on FRET systems with coupling of azo-derivatives of carbazole as a donor and spiropyran ethyl acrylate (SPEA) as an acceptor. In this study, monomers of an acrylic-modified spiropyran derivative (SPEA) and an azocarbazole derivative (AzoCzEA) were synthesized and then incorporated into the poly(methyl methacrylate) (PMMA) chains inside nanoparticles via emulsion polymerization. The obtained photoactive polymeric nanoparticles can protect dyes from environmental effects as internal conversion (IC), beside sustained photostability in long-term and photoreversibility with improving fatigue resistance. Finally, the observed complete fluorescence quenching of the donor moiety upon UV irradiation referred to the excellent overlap between the two chromophores in the obtained nanoparticles.

2. EXPERIMENTAL SECTION

2.1. Materials. 2,3,3-trimethylindolenine, and the surfactant sodium dodecyl sulfate (SDS, 99%) were purchased from Sigma-Aldrich. All of the solvents, 2-hydroxy-5-nitrobenzaldehyde, methyl methacrylate (MMA), potassium persulfate (KPS) as the initiator, sodium bicarbonate as a buffer ($NaHCO_3$), carbazole (Cz, 89%), sodium nitrite ($NaNO_2$), hydrochloric acid (HCl, 37%), 4-nitroaniline, triethylamine, Triton X-100, 2-bromoethanol, and acryloyl chloride (AC) were supplied by Merck Chemical Co. Tetrahydrofuran (THF) was dried over sodium and distilled off. Other solvents and all reagents were used without further purification. Deionized (DI) water was used in all recipes.

2.2. Synthesis of 1′-(2-Acryloxyethyl)-3′, 3′-dimethyl-6-nitro-spiro-(2-H-1-benzopyran-2,2′-indoline) (SPEA). 1′-(2-Acryloxyethyl)-3′, 3′-dimethyl-6-nitrospiro-(2-H-1-benzopyran-2,2′-indoline) (SPEA) was synthesized in four sequential steps according to the previously reported procedure by our group.[40] The forward synthesis of SPEA included: (1) Substitution nucleation reaction between 2,3,3-trimethylindolenine and 2-bromoethanol. (2) Conducting isomerization reaction of the product from step (1), resulting in (*R*/*S*)-9,9,9a-trimethyl-2,3,9,9a-tetrahydrooxazolo[3,2-*a*]indole (*R*/*S*). (3) Condensation reaction between the product of step (2) and 2-hydroxy-5-nitrobenzaldehyde, giving (*R*/*S*)-2-(3′,3′-dimethyl-6-nitro-3′*H*-spiro-[chromene-2,2′-indole]-1′-yl)ethanol (*R*/*S*) (SP−OH). (4) Modification of SP−OH with acryloyl chloride for preparation of SPEA (Figure 1a).

2.3. Synthesis of (4-Nitrophenyl)-[3-[*N*-[2-(acryloyloxy) ethyl] carbazolyl]] Diazene (AzoCzEA). (4-Nitrophenyl)-[3-[*N*-[2-(acryloyloxy)ethyl]carbazolyl]] diazene was synthesized based on the modified procedure reported before.[41] Primarily, *N*-(2-hydroxyethyl) carbazole (C-A) was synthesized by nucleation substitution reaction between carbazole with 2-bromoethanol in dimethyl formamid (DMF) in the presence of potassium hydroxide (KOH) at room temperature to give C-A (Figure 1b). After 12h, the reaction mixture was poured into water and the obtained white solid was filtered, washed with water, air-dried and finally purified by the modified recrystallization method from a 1:1 cyclohexane: toluene mixture to give white needle crystals with 88.7% yield. 3-[(4-Nitrophenyl)] azo-9*H*-carbazole-9-ethanol (NACzEtOH) was synthesized next. And finally, (4-nitrophenyl)-[3-[*N*-[2- (acryloyloxy) ethyl] carbazolyl]] diazene (AzoCzEA) was synthesized through the modification reaction of NACzEtOH with acryloyl chloride in dry THF by using of triethylamine as the catalyst. The resulting solid was purified by recrystallization from a 2:1 ethanol: chloroform mixture (in 83.4% yields).

Table 1. Solutions with Different Amounts of NACzEtOH and SP−OH[a]

sample	NACzEtOH (mg)	$C_{NACzEtOH}$ (mol/L)	SP−OH (mg)	C_{SP-OH} (mol/L)	$C_{SP-OH}/C_{NACzEtOH}$
DA1	0.2	1.85×10^{-5}	5.3	5.02×10^{-4}	27.1
DA2	0.4	3.71×10^{-5}	5.3	5.02×10^{-4}	13.5
DA3	0.8	7.42×10^{-5}	5.3	5.02×10^{-4}	6.8
DA4	1.6	1.48×10^{-4}	5.3	5.02×10^{-4}	3.4
DA5	3.3	3.06×10^{-4}	5.3	5.02×10^{-4}	1.6

[a] The volume was reached to 30 mL with acetone for each sample.

2.4. Preparation of Solutions Containing SP−OH and NACzEtOH. Several solutions with different amounts of NACzEtOH and SP−OH were prepared in acetone (Table 1). Then, their spectral features were investigated by UV−vis spectroscopy at 365 nm.

2.5. Emulsion Polymerization and Preparation of Dual-Color Fluorescent Polymeric Nanoparticles. The proportion of each component in preparation of different latexes has been given in Table 2. An aqueous solution of SDS (0.06 g) and Triton X-100 (0.03 g) as surfactants, $NaHCO_3$ (0.03 g) as the buffer and KPS (0.03 g) as the initiator was prepared, poured into a three-necked round-bottom reactor and purged with nitrogen gas. Afterword, the solution temperature reached to 70 °C. Then, solutions of SPEA in 5 mL of DI water and AzoCzEA in MMA (3.2 mL) were added from two separate funnels dropwise and simultaneously into the reactor within 20 min. Emulsion polymerization reaction continued for 3h, to reach to conversions of above 95%. The coagulation for each latex was less than 1 wt %.

2.6. Characterization. Identification of the synthesized products in every step was carried out by FT-IR BRUKER-IFS48 spectrophotometer (Germany), using KBr pellets. To approve the precise synthesis of azocarbazole ethyl acrylate, ^{1}HNMR spectrum was recorded on an Avance 250 MHz instrument (BRUKER, Germany) in $CDCl_3$ and using tetramethyl silane (TMS) as the internal reference. Characterization of the prepared copolymers was carried out by differential scanning calorimetry (DSC) under N_2 atmosphere at 10 °C/min heating rate by NETZSCH Instruments Co. (DSC 200, F3Maia, Germany; 30−150 °C). Also thermogravimetric analysis (TGA) was performed under N_2 atmosphere at 10 °C/min heating rate by TGA-STA: PL-1500 (UK) (30−600 °C). Dried latexes were used for these analyses directly. Particle size and size distribution were measured by using SEMATECH laser light scattering (LLS) (France). Size and morphology of the polymeric particles were characterized by SEM, Tescan Vega II (Czeck Republic). For this reason, a drop of the diluted latex was placed on a sample holder and dried under vacuum at 25 °C. Then, they were put under vacuum, evacuated, and a layer of gold was deposited under flushing with argon by using EMITECH K450x sputter-coating (England). Photochromic properties of the polymeric nanoparticles were investigated by UV−vis analysis via Shimadzu-UV2550 UV−vis Spectrophotometer (Japan) and the fluorescence behavior was studied by JASCO FP-6500 Spectrofluorometer (Japan). For these analyses, the initial latexes were diluted to 0.2 wt % for UV−vis spectroscopy and 1.0 wt % for fluorescence analysis. A UV lamp (365 nm), CAMAG 12VDC/VAC (50/60 Hz, 14VA, Switzerland), was used to stimulate changes in structure and absorption bands of the photochromic moieties and providing proper conditions for energy transfer between the two existing chromophores in the polymeric nanoparticles. Also the source for visible light was a common LED lamp with white light which was used for recovery of fluorescence of the donor and monitoring of photoreversibility in the nanoparticles. UV and vis irradiation times were set at 4 and 7 min, respectively, in normal excitation and isomerization for all the samples.

Table 2. Emulsion Polymerization and Preparation of Dual-Color Fluorescent Latexes with Various Amounts of the Chromophores

sample	SPEA (mg)	C_{SPEA} (mol/L)	azoCzEA(mg)	$C_{AzoCzEA}$ (mol/L)	$C_{SPEA}/C_{AzoCzEA}$
NP	0.0	0.0	0.0	0.0	0.0
NP1	30	2.46×10^{-3}	4.5	3.62×10^{-4}	6.8
NP2	60	4.92×10^{-3}	4.5	3.62×10^{-4}	13.5
NP3	90	7.4×10^{-3}	4.5	3.62×10^{-4}	20.4

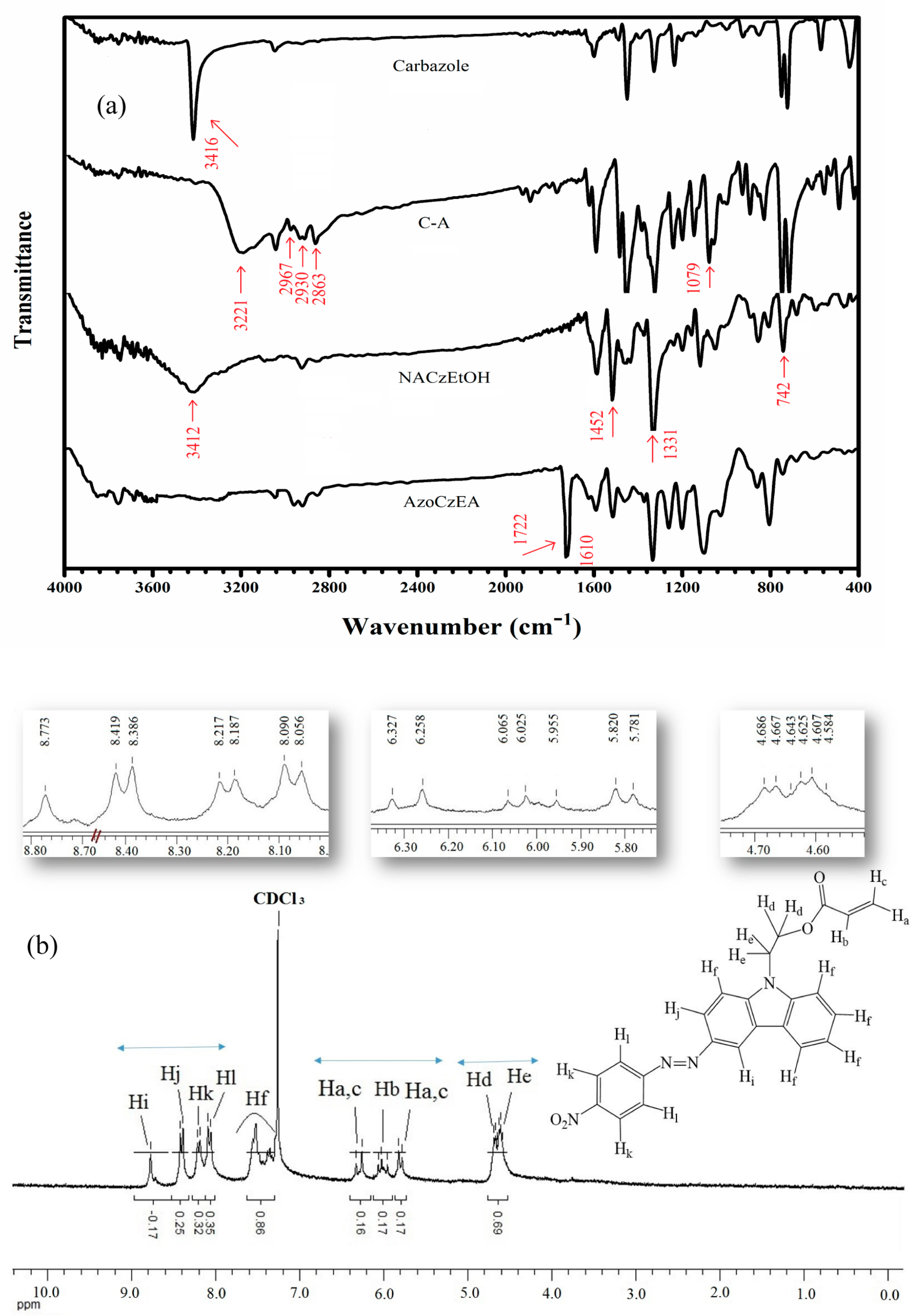


Figure 2. FT-IR spectra of the synthesized compounds in preparation of AzoCzEA (a) and ^{1}H NMR spectrum of AzoCzEA in CDCl$_3$ (the upper insets reveal the corresponding expanded regions) (b).

3. RESULTS AND DISCUSSION

One of the most promising employed strategies for preparation of the reversible photoswitchable dual-color nanoparticles is based on emulsion polymerization technique.[37] In this study, SP−OH and SPEA were synthesized according to our previous report.[40] C-A and NACzEtOH, as precursors in the synthesis of AzoCzEA, were prepared and purified according to the literature[41] with some modification in the method. Then, the new fluorescent dye (AzoCzEA) and SPEA were covalently incorporated into the PMMA polymeric matrix in the latex particles via emulsion polymerization. Such chemical attachment can efficiently avoid undesired effects like dye leakage and dye

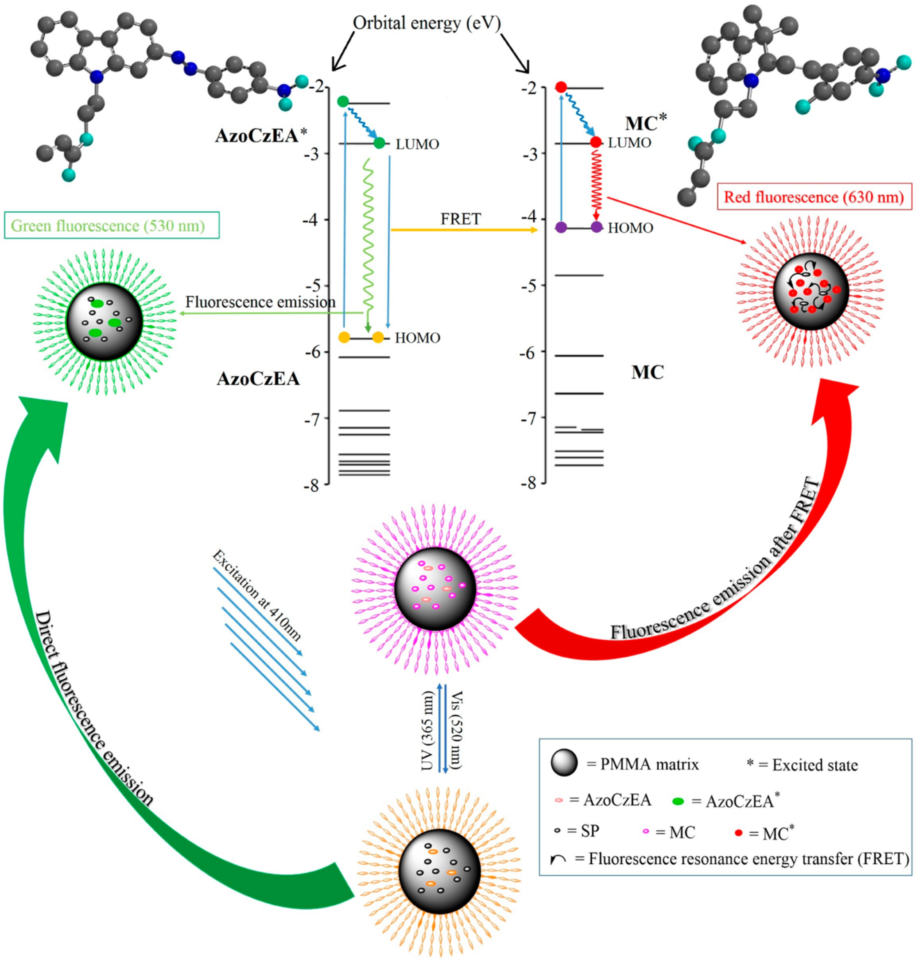


Figure 3. Schematic representation for illustrating radiative or nonradiative energy transfer between the AzoCzEA and SPEA at ground and excited states by UV irradiation.

aggregation, which occurs in physically doped polymeric systems and particles.[23,24]

3.1. Identification of AzoCzEA. At first, all synthesized compounds were characterized by FT-IR spectroscopy (Figure 2a). The synthesis of C-A was approved by the presence of characteristic peaks at 3221 cm^{-1}, 1079 cm^{-1} and 2863−2967 cm^{-1} relating to hydroxyl groups, C−O and C−H stretching vibrations of the aliphatic moieties, respectively. In addition, the elimination of N−H stretching vibration at 3416 cm^{-1} in carbazole ring confirms the progress of reaction. The shift of hydroxyl group stretching vibration in NACzEtOH to higher wavenumbers is due to the weak hydrogen bonding because of spatial repulsions. On the other hand, C−H bending vibration profile of carbazole at 712 and 742 cm^{-1} changed due to the substitution nucleation reaction on the carbazole ring with 4-nitrobenzenediazoniom chloride. Also symmetric and asymmetric stretching vibrations of NO_2 group at 1331 and 1452 cm^{-1} are another indication for the synthesis of NACzEtOH. AzoCzEA was obtained by esterification reaction of NACzEtOH. FT-IR spectrum of AzoCzEA demonstrates its characteristic peaks at 1722 and 1610 cm^{-1} that are related to the carbonyl and vinyl stretching vibrations respectively, beside the elimination of hydroxyl groups at 3412 cm^{-1}.

Figure 2b displays ^{1}HNMR spectrum of AzoCzEA with two doublets at 4.60 ppm (2H) and 4.67 ppm (2H), indicating methylene groups (H_d, H_e) attached to the nitrogen atom in the carbazole ring. The addition of acryloyloxy group was verified by the appearance of a doublet of doublet at 5.80 and 6.28 ppm (H_a, H_c) with a coupling constant of 123 Hz and a triplet at 6.02 ppm (H_b) relating to the vinylic protons. The protons of nitrobenzene ring (H_l and H_k) could be found as two doublets at 8.07 and 8.20 ppm. The proton of position 4 of the carbazole ring (H_i) has been appeared as a singlet at 8.77 ppm and also other protons of the carbazole ring are at 7.2−7.6 ppm. The results of ^{1}HNMR spectroscopy approve the modification of NACzEtOH with acryloyl chloride and successful synthesis of AzoCzEA. For better elucidation, some expanded regions have been given in the upper part of Figure 2b.

3.2. UV−Vis Analysis. Fluorescence quenching refers to any process which significantly reduces the fluorescence intensity of a given material. A variety of environmental factors can result in quenching, including energy transfer between fluorophores, interaction between the fluorophore and surrounding solvent molecules (dictated by solvent polarity), complex formation, collisional energy transfer, temperature, pressure, pH and localized concentration of the fluorescent species.[33,42,43] Mostly, the fluorescence intensity is influenced by the interactions with local environment in the excited state lifetime. The excited AzoCzEA fluorophore (AzoCzEA*) has two pathways to return to its ground state after excitation at 410 nm: (i) direct fluorescence emission, and (ii) nonradiation energy transfer to a nearest appropriate acceptor (Figure 3). SPEA (SP form) can

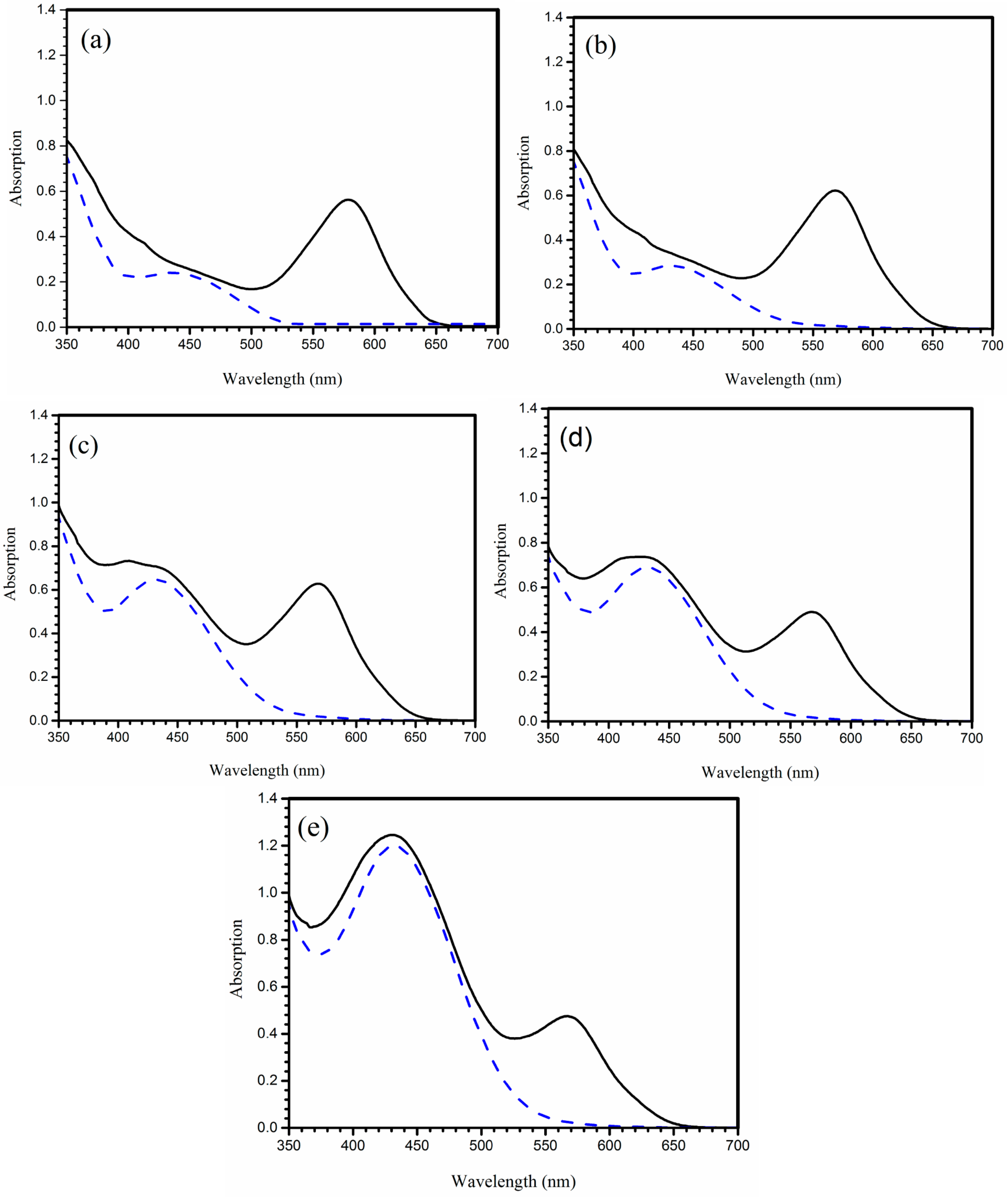


Figure 4. UV−vis analysis of DA1 (a), DA2 (b), DA3 (c), DA4 (d), and DA5 (e) solutions in acetone, before (- - -) and after (—) UV irradiation at 365 nm.

absorb UV irradiation at the 365 nm and isomerizes to its MC form. Then, MC isomer with absorption region of 500−650 nm will be a proper acceptor to receive energy from AzoCzEA* according to the significant band gap overlap and subsequently, quenching process occurs before the fluorescence emission of AzoCzEA at 530 nm. The orbital energy levels were calculated by Spartan'14, version 1.1.0.

Primarily, solutions of SP−OH/NACzEtOH in acetone (DA-series samples) were prepared and their UV−vis absorption spectra were recorded before and after irradiation at 365 nm to survey the possibility of spectral overlap and proper energy transfer between the above two chromophores (Figure 4).

NACzEtOH has an absorption band in 400−550 nm. When DA-series samples (Table 1) are irradiated at 365 nm, the spiropyran moiety will change from the closed-ring isomer (spiro, SP) to the open-ring one (merocyanine, MC) with a strong absorption band at 400−700 nm (λ_{max} of 560 nm). SP−OH solution shows no absorption band before irradiation at this region at all. The emission band of NACzEtOH lay in the absorption region of MC isomer. Hence, there will be the possibility of efficient overlap between MC absorption and NACzEtOH emission. It provides the situation for quenching of the fluorescent dye and correspondingly, the relevant absorption at 410 nm will be disappeared. This could be observed for DA1, DA2 and almost DA3 (Figure 4, parts a−c). But the quenching phenomenon was not monitored for DA4 and DA5 (Figure 4, parts d and e). It is noteworthy that the quenching process depends on the concentration ratio of acceptor to donor in dual-color polymeric systems.[24] To have maximum quenching yield, it is necessary that some acceptor chromophores surround each donor and proceed this process sufficiently. The weak quenching in DA4 and DA5 could be attributed to the high concentration of donors (NACzEtOH) and lack of enough MC isomer as the acceptor (quenching agent) in these solutions. Complete quenching was observed for DA1 and DA2, but the absorption intensity at 500−650 nm for DA2 is more than DA1 after irradiation at 365 nm, owing to the higher energy transfer from NACzEtOH to SP−OH in DA2. The comparison between DA2 and DA3 illustrates that despite similar absorption intensities (500−650 nm), the complete quenching (400−550 nm) has been accomplished for DA2. Therefore, DA2 with $C_{SP-OH}/C_{NACzEtOH}$ of 13.5 was chosen as the optimum sample with the best quenching and synergistic effect for observing FRET phenomenon.

3.3. Dual-Color Fluorescent Polymeric Nanoparticles.

Here, the employed basic strategy for preparation of the novel photoswitchable fluorescent polymeric nanoparticles was based on emulsion polymerization. All of the nanoparticles were made of SPEA−AzoCzEA−MMA copolymer chains. AzoCzEA (the fluorescent dye) serves as the electron donor and SPEA (the photochromic dye) acts as the electron acceptor moiety and both are covalently bound to MMA in polymeric chains in the nanoparticles (Figure 5).

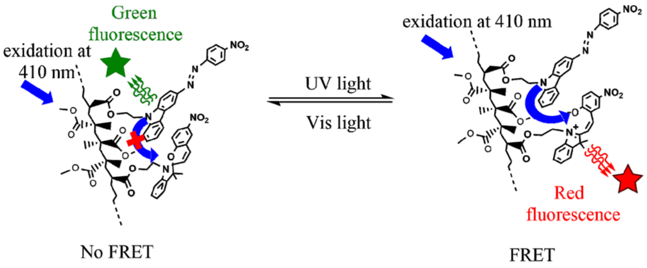


Figure 5. Schematic representation of a SPEA−AzoCzEA−MMA copolymer chain and FRET process accompanied by fluorescence color change through UV−vis irradiation.

Covalently linked dyes and the polymer chains effectively avoid dye leakage and remove the possibility of dye aggregation in long-term, which are undesirable features in the noncovalently introduced dyes into the polymeric matrix. To have an optimum FRET phenomenon, in addition to photoluminescence properties and good overlap between donor and acceptor components in the nanoparticles,[24,37,44] particles size should reach to less than 100 nm.[40] This could be attributed to the high scattering of light beams by the particles, while they are equal or larger than half of the incident light wavelength (λ/2). The strength point in emulsion polymerization is the capability to tune particles size by variation of some parameters such as surfactants concentration or ionic strength of the polymerization environment.

SPEA and AzoCzEA with identical molar ratios in DA2 were copolymerized with MMA through emulsion polymerization to give dual-color fluorescent nanoparticles. Typically, the morphology and size distribution of photoswitchable fluorescent NP2 nanoparticles were studied by scanning electron microscopy (SEM) and dynamic light scattering (DLS) (Figure 6).

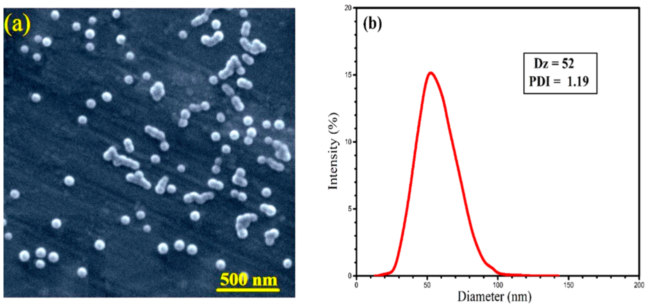


Figure 6. Particle size and size distribution for NP2 dual-color fluorescent nanoparticles by SEM (a) and DLS (b) analyses.

Both show a good agreement and particles with narrow size distribution (40−80 nm) have been obtained. SEM micrograph reveals the uniform spherical nanoparticles with a smooth surface and narrow size distribution. Also DLS analysis reveals a unimodal pattern and confirms the successive progress of emulsion polymerization with one regulating nucleation mechanism without existence of secondary particles.

UV−vis analysis of NP2 latex demonstrates a complete quenching upon UV irradiation (Figure 7). In order to confirm the previous results in the solution state for determining optimum ratio of the two chromophores, NP1 with less and NP3 with higher ratios relative to NP2 were prepared and observed too. Disappearance of the absorption band at 400−550 nm after UV irradiation in NP2 is quite evident here. The band appeared at 350−400 nm after UV irradiation (365 nm) is attributed to the $\pi \rightarrow \pi^*$ electronic transition of the chromene moiety. The highest absorption intensity at 500−650 nm for NP2 approves the obtained optimized ratio for energy transfer and synergistic effect between these two chromophores. This was the starting point for our extensive studies on such dual-color polymer particles and the results will be discussed in the upcoming.

3.4. Thermal Properties.

Thermal properties of PMMA (NP) and NP2 sample containing AzoCzEA and SPEA were investigated by differential scanning calorimetry and thermogravimetric analyses (Figure 8). A comparative study of glass transition temperature (T_g) by DSC depicts an increment in T_g from 102 °C for NP to 116 °C for NP2 as a result of incorporation of AzoCzEA and SPEA into the polymeric chains in the nanoparticles. The DSC thermogram of NP2 indicates a single transition temperature at 116 °C that has a few raise with respect to the amorphous structure of PMMA and could be attributed to the incorporation of AzoCzEA and SPEA as comonomers into the PMMA polymeric matrix. Introducing these bulk chromophores as comonomers to the polymer backbone leads to an increase in the restriction of mobility and steric hindrance of PMMA chains. It is worth mentioning that no phase separation was observed here according to the appearance of single transition temperature in DSC thermogram (Figure 8a).

Thermal resistance of the prepared latex nanoparticles was studied by TGA. Figure 8b indicates that thermal stability of the

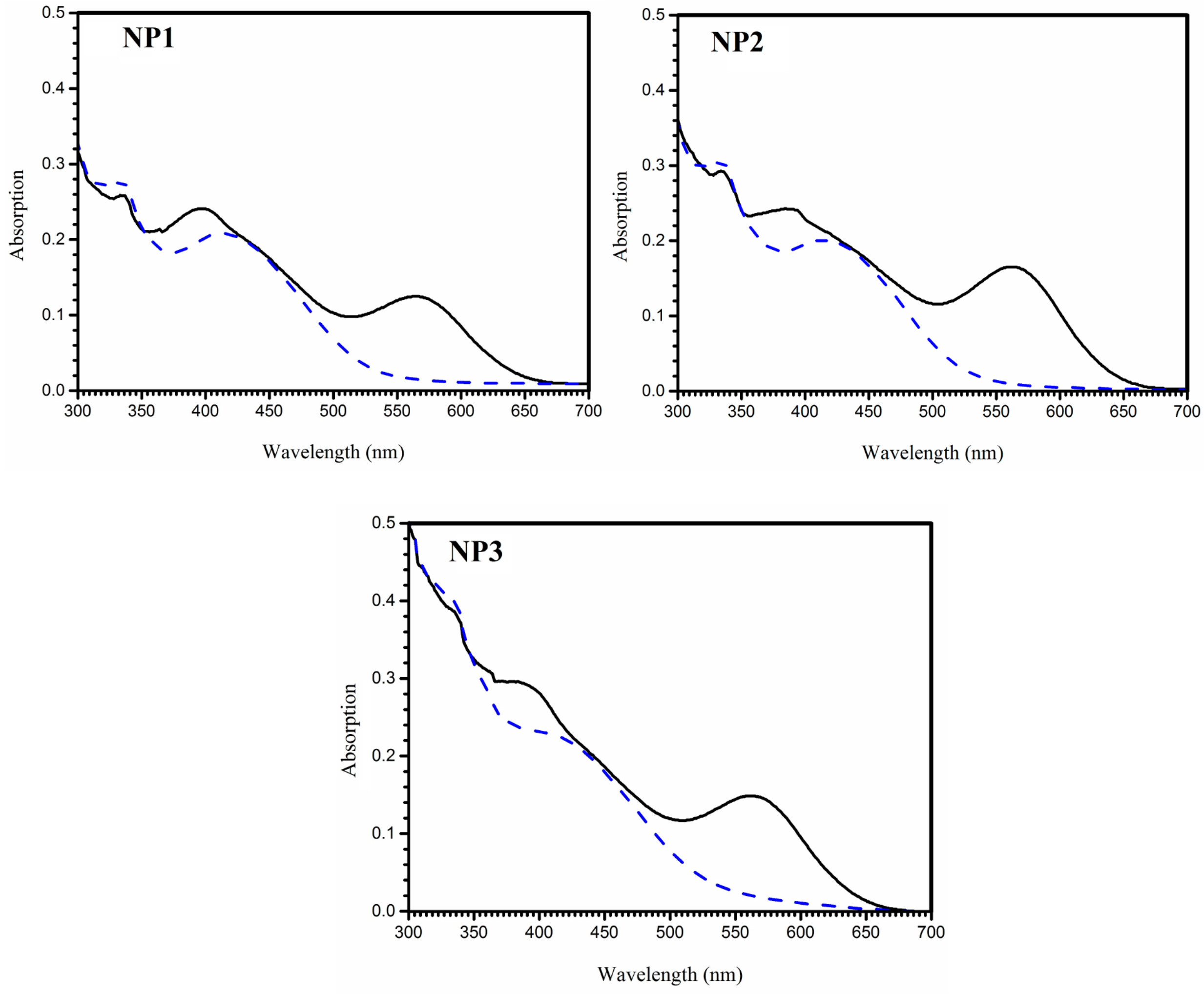


Figure 7. UV−vis analysis of the latexes containing dual-color fluorescent polymeric nanoparticles before (- - -) and after (—) UV irradiation at 365 nm.

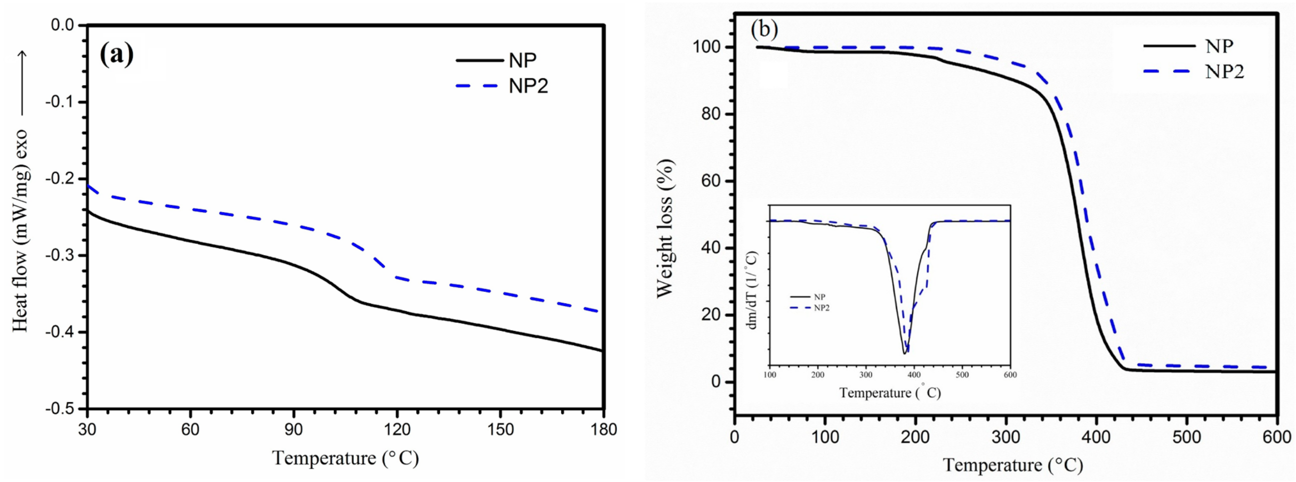


Figure 8. DSC (a) and TGA (b) thermograms of NP and NP2 samples (the inset in TGA thermogram shows corresponding DTGs).

copolymer containing two chromophores shows a slight increase with respect to PMMA polymeric matrix because of the presence of aromatic structures. It should be noted that both samples degrade in similar single-step and profile. A multistep decomposition and different degradation pattern would be expectable, if SPEA and/or AzoCzEA generate their own aggregates during polymerization. Totally, the observations from DSC and TGA thermograms imply that SPEA and AzoCzEA have been successfully copolymerized with MMA and distributed in the copolymer chains appropriately.

DOI: 10.1021/acs.macromol.5b02401
Macromolecules XXXX, XXX, XXX−XXX

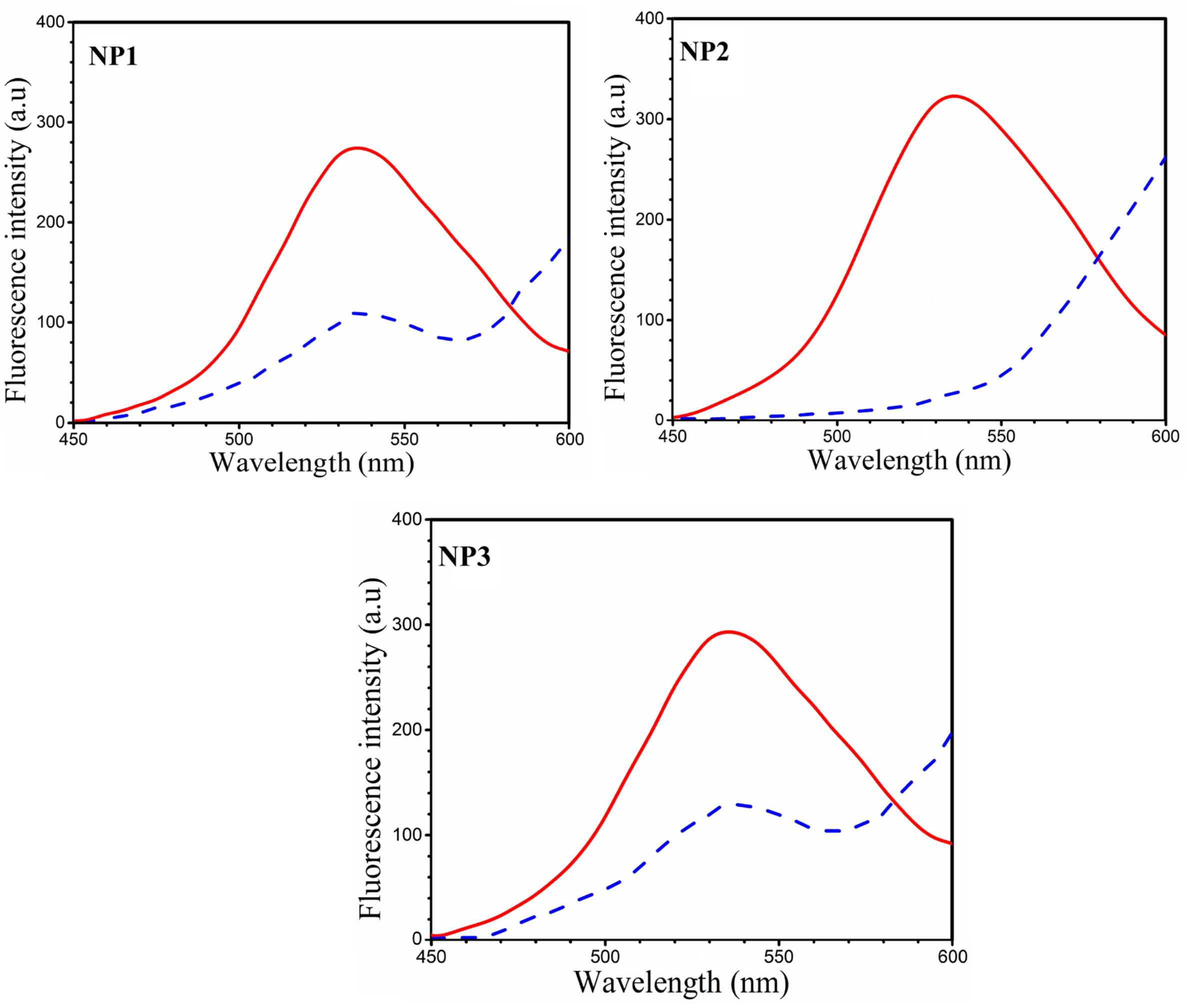


Figure 9. Fluorescence spectroscopy of the prepared dual-color latexes before (—) and after (- - -) UV irradiation at 365 nm for 4 min (the samples were preirradiated at 410 nm).

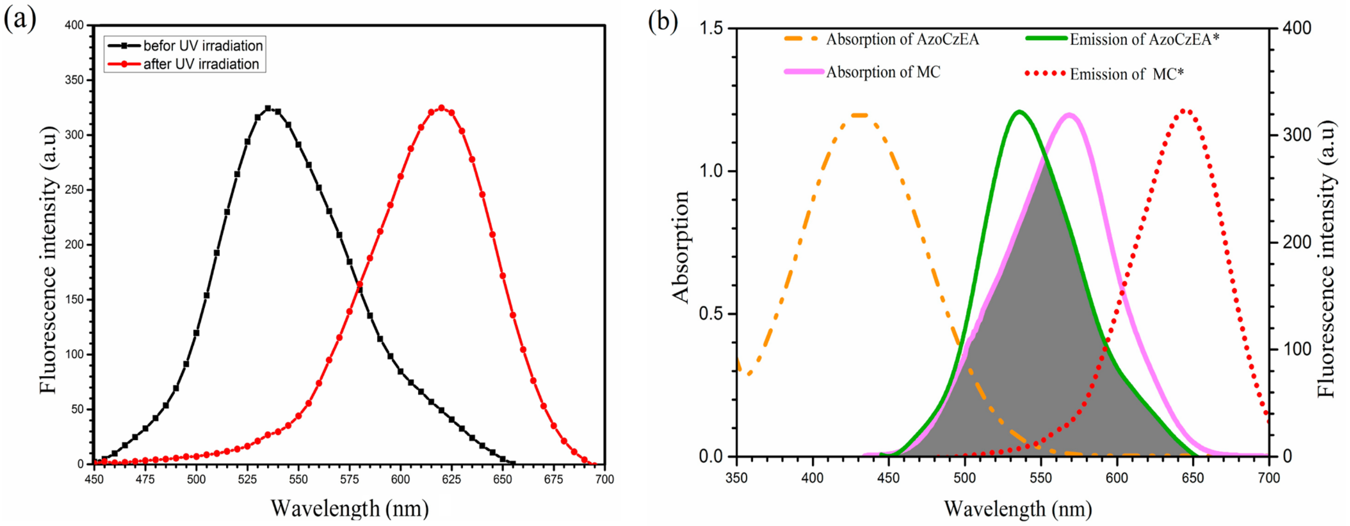


Figure 10. (a) Fluorescence emission spectra of NP2 dual-color fluorescence polymeric nanoparticles in water (excited at 410 nm), before and after UV irradiation at 365 nm. (b) Schematic representation of the absorption and fluorescence spectra of AzoCzEA and MC chromophores (the gray area indicates the spectral overlap between fluorescence emission of AzoCzEA* as the donor and absorption band of MC as the acceptor).

3.5. Fluorescence and Photoreversibility of the Polymeric Nanoparticles. As mentioned before, merocyanine isomer of SPEA has a fluorescence emission at λ_{max} of 630 nm after excitation at 550 nm. Therefore, a reasonable overlap

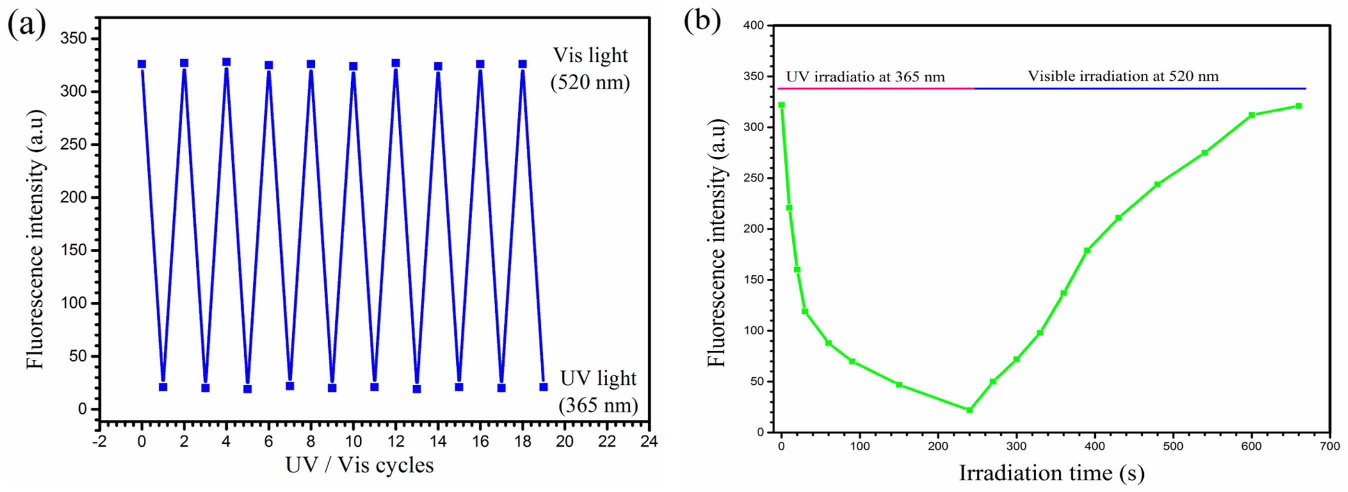


Figure 11. Reversible fluorescence photoswitching of AzoCzEA-SPEA in NP2 latexes upon UV and visible irradiations for 10 cycles (a) and variation in fluorescence intensity for a cycle of UV (4 min) and visible (7 min) irradiation (b).

between fluorescence emission of AzoCzEA and absorption of MC isomer will result in a nonradiation energy transfer from the excited AzoCzEA to MC and subsequent fluorescence emission of MC at 630 nm just by excitation at 410 nm.

The analysis on DA2 sample did not show any fluorescence emission for NACzEtOH and SP−OH in acetone solution, although UV−vis spectroscopy (Figure 4) revealed the possibility of overlap between these two chromophores. This could be attributed to the nonradiative decay of the excited states (SP−OH* and/or NACzEtOH*) by internal conversion (IC) processes that were discussed in the preceding. These ICs predominate over the radiative processes, causing insignificant fluorescence emission. Inclusion of these moieties into a polymeric matrix would be a good resolution to suppress these IC processes and observing preferred radiative emissions. In other words, the probable ICs in solution would become less probable while entrapped in a polymeric phase. This is a great privilege for immobilization of these chromophores into a polymeric matrix and removing internal conversions possibility. Among polymeric systems, nanoparticles are of high importance owing to their dispersibility in diverse media and having these dyes in the liquid state (specifically in water phase for hydrophobic dyes) and observing their radiation-emission responses.

In this work and with respect to the above approach, the prementioned polymeric latex nanoparticles were prepared to restrict dye leaching and aggregation and also ICs. These particles exhibited good structural stability, photostability and excellent switching. Fluorescence spectroscopy of the prepared dispersed nanoparticles in water was recorded and shown in Figure 9. They showed a green emission at 530 nm after excitation at 410 nm. Upon UV irradiation at 365 nm for 4 min, fluorescence intensity of the dual-color nanoparticles reduced remarkably. This is due to the occurrence of SPEA isomerization, which results in nonradiative energy transfer between AzoCzEA* to MC isomer.

Fluorimetry analysis approved that NP2 sample with an optimized ratio of the two chromophores shows maximum energy transfer with complete quenching after UV irradiation at 365 nm for 4 min. Hence, the fluorescence red emission of MC at 630 nm was observed (Figure 10a). Totally, red and green fluorescence emissions are visible reversibly with switching UV light on and off at 365 nm, respectively after excitation at 410 nm.

Figure 10b demonstrates the overlap between AzoCzEA fluorescence emission and MC absorption in the prepared polymeric nanoparticles in 450−700 nm schematically. Another possibility would be providing the appropriate situation for good stacking between carbazole and spiropyran rings in the polymeric nanoparticles that triggers FRET process efficiently.

Photoswitchable nature of the fluorescent NP2 nanoparticles has been shown in Figure 11 upon exposure to alternating illumination cycles of UV (365 nm, 4 min) and vis (520 nm, 7 min) lights. Diluted NP2 latex with 1 wt % solid content was irradiated with repeated cycles of UV−vis lights and in each cycle. The sample was excited at 410 nm and the fluorescence intensity was recorded at 450−600 nm. This process is due to the optically switching and conversion between SP and MC by using alternating irradiation at 365 and 520 nm in which, the fluorescence intensity of AzoCzEA is switched "off" and "on" in these nanoparticles, respectively. Our investigations depict that the incorporation of both SPEA and AzoCzEA chromophores into the hydrophobic polymeric matrix by covalent linkage substantially enhances the photoreversibility beside photostability in these nanoparticles. These imply that light-induced reversibility of fluorescence emission can be repeated several times (Figure 11a) with remarkable fatigue resistance for long time without any leaching with respect to other nonbonded dyes in polymers. Typically, the variations in fluorescence intensity with time upon UV irradiation at 365 nm and also visible irradiation at 520 nm in a cycle have been given in Figure 11b.

4. CONCLUSION

In this study, preparation and characterization of new dual-color fluorescent polymeric nanoparticles with average particle size of 40−80 nm were discussed by means of emulsion polymerization in the aqueous phase. An acrylic azocarbazole derivative (AzoCzEA) was synthesized and copolymerized with MMA and an acrylic-functionalized spiropyran (SPEA) and corresponding FRET process was investigated in the obtained stimuli responsive photoreversible nanoparticles. AzoCzEA was used as the fluorescent (donor) moiety and SPEA was exploited as the photochromic (acceptor) group. At certain conditions and ratios, the intermolecular interactions between light absorbing donor and energy receiving acceptors were found to be the main responsible phenomenon for energy transfer. Hence, the fluorescence and UV−vis analysis showed that the fluorescence emission of AzoCzEA (λ_{max} = 530 nm) overlapped appropriately with the absorption band of SPEA (λ_{max} of 550 nm after UV irradiation at 365 nm). The optimized NP2 latex revealed a green emission at 530 nm after excitation at 410 nm. Upon UV irradiation at 365 nm, the above green fluorescence emission was quenched and a red fluorescence at 630 nm was observed. Maximum efficiency of nonradiative energy transfer for quenching of the fluorescence emission was obtained the in these dual-color fluorescent nanoparticles. The covalent bonding between AzoCzEA, SPEA, and MMA in the polymeric matrix was identified by thermal analysis and this was responsible for the structural stability and preventing dye leakage as the main concern in such systems. However, internal conversions with surrounding environment were controlled by inclusion of the chromophores and their fixation in the polymer. Investigations on reversibility and fluorescence photoswitching of the prepared dual-color latexes depict their prominent photostability and excellent fatigue resistance with no leaching and aggregation of the chromophores. These dual-color photoswitchable fluorescent nanoparticles could be exploited in biological systems such cell labeling and cell imaging with fluorescence tracing capability, rewriteable patterning, and photosensitive displays.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +9821 4478 7000. Fax: +9821 4478 7023. E-mail: a.mahdavian@ippi.ac.ir.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We wish to express our gratitude to Iran Polymer and Petrochemical Institute (IPPI) for financial support of this work (Grant No. 24761172).

■ REFERENCES

(1) Chen, J.; Zhang, P.; Fang, G.; Yi, P.; Yu, X.; Li, X.; Zeng, F.; Wu, Sh. Synthesis and characterization of novel reversible photoswitchable fluorescent polymeric nanoparticles via one-step miniemulsion polymerization. *J. Phys. Chem. B* **2011**, *115*, 3354−3362.

(2) Wang, Y.; Hong, C. H.; Pan, C. P. Spiropyran-based hyperbranched star copolymer: synthesis, phototropy, FRET, and bioapplication. *Biomacromolecules* **2012**, *13*, 2585−2593.

(3) Sennett, K. A.; Lindner, B. K.; Kaur, N.; Fetner, S. M.; Stitzel, S. E. Synthesis and photochromic properties of methacryloxy 6-nitrospirobenzopyrans. *Dyes Pigm.* **2013**, *98*, 437−441.

(4) Beyer, C.; Wagenknecht, H. A. Synthesis of spiropyrans as building blocks for molecular switches and dyads. *J. Org. Chem.* **2010**, *75*, 2752−2755.

(5) Chen, J.; Zhong, W.; Tang, Y.; Wu, Z.; Li, Y.; Yi, P.; Jiang, j. Amphiphilic BODIPY-Based Photoswitchable Fluorescent Polymeric Nanoparticles for Rewritable Patterning and Dual-Color Cell Imaging. *Macromolecules* **2015**, *48*, 3500−3508.

(6) Achilleos, D. S.; Vamvakaki, M. Multiresponsive spiropyran-based copolymers synthesized by atom transfer radical polymerization. *Macromolecules* **2010**, *43*, 7073−7081.

(7) Son, S.; Shin, E.; Kim, B.-S. Light-responsive micelles of spiropyran initiated hyperbranched polyglycerol for smart drug delivery. *Biomacromolecules* **2014**, *15*, 628−634.

(8) Ming, L.; Gu, L. Y.; Zhang, Q.; Xue, M. Z.; Liu, Y. G. Preparation and study of photoswitchable fluorescence nanoparticles based on spirobenzopyran. *Chin. Chem. Lett.* **2013**, *24*, 1014−1018.

(9) Zhu, M.; Zhu, L.; Han, J. J.; Wu, W.; Hurst, J. K.; Li, A. D. Q. Spiropyran-Based Photochromic Polymer Nanoparticles with Optically Switchable Luminescence. *J. Am. Chem. Soc.* **2006**, *128*, 4303−4309.

(10) Lv, Y.; Liu, H.; Zhao, B.; Tian, Z.; Li, A. D. Q. Tuning Photoswitchable Dual-Color Fluorescence from Core-Shell Polymer Nanoparticles. *Isr. J. Chem.* **2013**, *53*, 294−302.

(11) Tian, W.; Tian, J. An insight into the solvent effect on photo-, solvato-chromism of spiropyran through the perspective of intermolecular interactions. *Dyes Pigm.* **2014**, *105*, 66−74.

(12) Liu, Y.; Wu, J.; Meng, L.; Zhang, L.; Lu, X. Self-assembled, fluorescent polymeric micelles of a graft copolymer containing carbazole for thermo-controlled drug delivery in vitro. *J. Biomed. Mater. Res., Part B* **2008**, *85B*, 435−443.

(13) Chang, C.-C.; Kuo, I.-C.; Ling, I.-F.; Chen, C.-T.; Chen, H.-C.; Lou, P.-J.; Lin, J.-J.; Chang, T.-C. Detection of quadruplex DNA structures in human telomeres by a fluorescent carbazole derivative. *Anal. Chem.* **2004**, *76*, 4490−4494.

(14) Palayangoda, S. S.; Cai, X.; Adhikari, R. M.; Neckers, D. C. Carbazole-based donor-acceptor compounds: highly fluorescent organic nanoparticles. *Org. Lett.* **2008**, *10*, 281−284.

(15) van Dijken, A.; Bastiaansen, J. J. A. M.; Kiggen, N. M. M.; Langeveld, B. M. W.; Rothe, C.; Monkman, A.; Bach, I.; Stossel, P.; Brunner, K. Carbazole Compounds as Host Materials for Triplet Emitters in Organic Light-Emitting Diodes: Polymer Hosts for High-Efficiency Light-Emitting Diodes. *J. Am. Chem. Soc.* **2004**, *126*, 7718−7727.

(16) Yoon, S.-J.; Chun, H.; Lee, M.-S.; Kim, N. Preparation of poly (N-vinylcarbazole) (PVK) nanoparticles by emulsion polymerization and PVK hollow particles. *Synth. Met.* **2009**, *159*, 518−522.

(17) Morin, J.-F.; Leclerc, M. Syntheses of conjugated polymers derived from N-alkyl-2, 7-carbazoles. *Macromolecules* **2001**, *34*, 4680−4682.

(18) Tsutsumi, N.; Kinashi, K.; Ogo, K.; Fukami, T.; Yabuhara, Y.; Kawabe, Y.; Tada, K.; Fukuzawa, K.; Kawamoto, M.; Sassa, T.; Fujihara, T.; Sasaki, T.; Naka, Y. Updatable Holographic Diffraction of Monolithic Carbazole-Azobenzene Compound in Poly (methyl methacrylate) Matrix. *J. Phys. Chem. C* **2015**, *119*, 18567−18572.

(19) Grazulevicius, J. V.; Strohriegl, P.; Pielichowski, J.; Pielichowski, K. Carbazole-containing polymers: synthesis, properties and applications. *Prog. Polym. Sci.* **2003**, *28*, 1297−1353.

(20) Zhang, B.; Chen, Y.; Zhuang, X.; Liu, G.; Yu, B.; Kang, E.-T.; Zhu, J.; Li, Y.L. Poly (N-vinylcarbazole) chemically modified graphene oxide. *J. Polym. Sci., Part A: Polym. Chem.* **2010**, *48*, 2642−2649.

(21) Adhikari, R. M.; Mondal, R.; Shah, B. K.; Neckers, D. C. Synthesis and photophysical properties of carbazole-based blue light-emitting dendrimers. *J. Org. Chem.* **2007**, *72*, 4727−4732.

(22) Liu, Y.; Nishiura, M.; Wang, Y.; Hou, Z. π-Conjugated aromatic enynes as a single-emitting component for white electroluminescence. *J. Am. Chem. Soc.* **2006**, *128*, 5592−5593.

(23) Chen, J.; Zeng, F.; Wu, S.; Chen, Q.; Tong, Z. A core-shell nanoparticle approach to photoreversible fluorescence modulation of a hydrophobic dye in aqueous media. *Chem. - Eur. J.* **2008**, *14*, 4851−4860.

(24) Chen, J.; Zeng, F.; Wu, S.; Su, J.; Tong, Z. Photoreversible Fluorescent Modulation of Nanoparticles via One-Step Miniemulsion Polymerization. *Small* **2009**, *5*, 970−978.

(25) Li, C.; Zhang, Y.; Hu, J.; Cheng, J.; Liu, S. Reversible three-state switching of multicolor fluorescence emission by multiple stimuli modulated FRET processes within thermoresponsive polymeric micelles. *Angew. Chem., Int. Ed.* **2010**, *49*, 5120−5124.

(26) Zadran, S.; Standley, S.; Wong, K.; Otiniano, E.; Amighi, A.; Baudry, M. Fluorescence resonance energy transfer (FRET)-based biosensors: visualizing cellular dynamics and bioenergetics. *Appl. Microbiol. Biotechnol.* **2012**, *96*, 895−902.

(27) Cheng, P.-C. The contrast formation in optical microscopy in *Handbook of Biological Confocal Microscopy*, 3rd ed.; Pawley, J. B., Ed.; Springer: New York, 2006; pp 162−206.

DOI: 10.1021/acs.macromol.5b02401
Macromolecules XXXX, XXX, XXX−XXX

(28) Rajdev, P.; Basak, D.; Ghosh, S. Insights into Noncovalently Core Cross-Linked Block Copolymer Micelles by Fluorescence Resonance Energy Transfer (FRET) Studies. *Macromolecules* **2015**, *48*, 3360−3367.

(29) Roy, R.; Hohng, S.; Ha, T. A practical guide to single-molecule FRET. *Nat. Methods* **2008**, *5*, 507−516.

(30) Helms, V. *Principles of Computational Cell Biology*; Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany, 2008.

(31) Clegg, R. M. Fluorescence resonance energy transfer. *Curr. Opin. Biotechnol.* **1995**, *6*, 103−110.

(32) Zheng, J. Spectroscopy-based quantitative fluorescence resonance energy transfer analysis. *Ion Channels* **2006**, *337*, 65−77.

(33) Zhu, M.-Q.; Zhang, G.-F.; Hu, Z.; Aldred, M. P.; Li, C.; Gong, W.-L.; Chen, T.; Huang, Z.-L.; Liu, s. Reversible Fluorescence Switching of Spiropyran-Conjugated Biodegradable Nanoparticles for Super-Resolution Fluorescence Imaging. *Macromolecules* **2014**, *47*, 1543−1552.

(34) Ando, R.; Mizuno, H.; Miyawaki, A. Regulated fast nucleocytoplasmic shuttling observed by reversible protein highlighting. *Science* **2004**, *306*, 1370−1373.

(35) Wiedenmann, J.; Ivanchenko, S.; Oswald, F.; Schmitt, F.; Röcker, C.; Salih, A.; Spindler, K.-D.; Nienhaus, G. U. EosFP, a fluorescent marker protein with UV-inducible green-to-red fluorescence conversion. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 15905−15910.

(36) Chudakov, D. M.; Verkhusha, V. V.; Staroverov, D. B.; Souslova, E. A.; Lukyanov, S.; Lukyanov, K. A. Photoswitchable cyan fluorescent protein for protein tracking. *Nat. Biotechnol.* **2004**, *22*, 1435−1439.

(37) Zhu, L.; Wu, W.; Zhu, M.-Q.; Han, J. J.; Hurst, J. K.; Li, A. D. Q. Reversibly photoswitchable dual-color fluorescent nanoparticles as new tools for live-cell imaging. *J. Am. Chem. Soc.* **2007**, *129*, 3524−3526.

(38) Wang, S.; Yu, C.; Choi, M.; Kim, S. Synthesis and switching properties of photochromic carbazole − spironaphthoxazine copolymer. *J. Photochem. Photobiol., A* **2007**, *192*, 17−22.

(39) Bertarelli, C.; Bianco, A.; Castagna, R.; Pariani, G. Photochromism into optics: Opportunities to develop light-triggered optical elements. *J. Photochem. Photobiol., C* **2011**, *12*, 106−125.

(40) Abdollahi, A.; Mahdavian, A. R.; Salehi-Mobarakeh, H. Preparation of stimuli-responsive functionalized latex nanoparticles. The effect of spiropyran concentration on size and photochromic properties. *Langmuir* **2015**, *31*, 10672−10682.

(41) Ho, M. S.; Barrett, C.; Paterson, J.; Esteghamatian, M.; Natansohn, A.; Rochon, P. Synthesis and optical properties of poly {(4-nitrophenyl)-[3-[N-[2-(methacryloyloxy) ethyl]-carbazolyl]] diazene}. *Macromolecules* **1996**, *29*, 4613−4618.

(42) Valeur, B., Berberan-Santos, M. N. *Molecular fluorescence: principles and applications*, 2nd ed.; Wiley-VCH Verlag & Co, KGaA: Weinheim, Germany, 2012.

(43) Zhu, M. Q.; Zhang, G. F.; Li, C.; Aldred, M. P.; Chang, E.; Drezek, R. a.; et al. Reversible two-photon photoswitching and two-photon imaging of immunofunctionalized nanoparticles targeted to cancer cells. *J. Am. Chem. Soc.* **2011**, *133*, 365−372.

(44) Raymo, M. F.; Tomasulo, M. Electron and energy transfer modulation with photochromic switches. *Chem. Soc. Rev.* **2005**, *34*, 327−336.

DOI: 10.1021/acs.macromol.5b02401
Macromolecules XXXX, XXX, XXX−XXX